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(54) Title: HYDRAZINO-TYPE RADIONUCLIDE CHELATORS HAVING AN N₃S CONFIGURATION

(57) Abstract

Radionuclide chelating compounds are provided for conjugation to targeting molecules such as proteins, peptides or antibodies. The resulting labelled targeting molecules may be used in diagnosis and therapy.

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Hydrazino-type Radionuclide Chelators Having an N,S Configuration

Field of the Invention

5 This invention is in the field of diagnostic imaging, and relates to chemical chelators useful in the radiolabelling of agents that target tissues of diagnostic interest.

10 Background to the Invention

The art of diagnostic imaging exploits contrasting agents that in binding or localizing site selectively within the body, help to resolve the image of diagnostic interest. $^{67}\text{Gallium}$ salts, for example, have an affinity 15 for tumours and infected tissue and, with the aid of scanning tomography, can reveal afflicted body regions to the physician. Other contrasting agents include the metal radionuclides such as $^{99\text{m}}\text{technetium}$ and $^{186/188}\text{rhenium}$, and these have been used to label targeting molecules, 20 such as proteins, peptides and antibodies that localize at desired regions of the human body.

As targeting agents, proteins and other macromolecules can offer the tissue specificity required for diagnostic accuracy; yet labelling of these agents with metal 25 radionuclides is made difficult by their physical structure. Particularly, protein and peptide targeting agents present numerous sites at which radionuclide binding can occur, resulting in a product that is labelled heterogeneously. Also, and despite their 30 possibly large size, proteins rarely present the structural configuration most appropriate for high affinity radionuclide binding, i.e. a region incorporating four or more donor atoms that form five-membered rings. As a result, radionuclides are bound 35 typically at the more abundant low-affinity sites, forming unstable complexes. To deal with the problem of background binding, Paik et al (Nucl Med Biol 1985, 12:3)

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proposed a method whereby labelling of antibody is performed in the presence of excess DPTA (diaminetrimethylenepentaacetic acid), to mask the low affinity binding sites. While the problem of low 5 affinity binding is alleviated by this method, actual binding of the radionuclide, in this case technetium, was consequently also very low. The direct labelling of proteins having a high proportion of cysteine residues also has been demonstrated (Dean et al; WO 92/13,572).
10 This approach exploits thiol groups of cysteine residues as high-affinity sites for radionuclide binding, and is necessarily limited in application to those targeting agents having the required thiol structure.

A promising alternative to the direct labelling of 15 targeting agents is an indirect approach, in which targeting agent and radionuclide are conjugated using a chelating agent. Candidates for use as chelators are those compounds that bind tightly to the chosen metal radionuclide and also have a reactive functional group 20 for conjugation with the targeting molecule. For use in labelling peptide and protein-based targeting agents, the chelator is ideally also peptide-based, so that the chelator/targeting agent conjugate can be synthesized *in toto* using peptide synthesis techniques. For utility in 25 diagnostic imaging, the chelator desirably has characteristics appropriate for its *in vivo* use, such as blood and renal clearance and extravascular diffusibility.

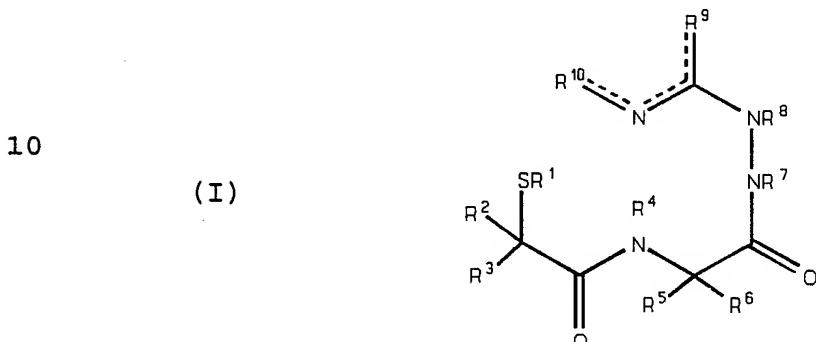
30 Summary of the Invention

The present invention provides chelators that bind diagnostically useful metal radionuclides, and can be conjugated to targeting agents capable of localizing at body sites of diagnostic and therapeutic interest. The 35 chelators of the present invention are peptide analogs designed structurally to present an N₃S configuration capable of binding oxo, dioxo and nitrido ions of

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^{99m}technetium and ^{186/188}rhenium.

More particularly, and according to one aspect of
the invention, there are provided metal radionuclide
5 chelators of the formula:



wherein

R¹ is H or a sulfur protecting group;

R² and R³ are selected independently from H; carboxyl;

lower alkyl; and lower alkyl substituted with a
group selected from hydroxyl, sulfhydryl, halogen,
carboxyl and aminocarbonyl;

R⁴ and R⁷ are each H;

R⁵ and R⁶ are selected independently from H; carboxyl;
lower alkyl; lower alkyl substituted with a group
selected from hydroxyl, sulfhydryl, halogen,
carboxyl, lower alkoxy carbonyl and aminocarbonyl;
and an alpha carbon side chain of any amino acid
other than proline;

R⁸ is selected from H, carboxyl, lower alkyl and lower
alkyl substituted with hydroxyl, carboxyl or
halogen; and

R⁹ and R¹⁰ together form a 5- or 6-membered, saturated or
unsaturated heterocyclic ring which is optionally
fused to another 5- or 6-membered saturated or
unsaturated, heterocyclic or carbocyclic ring
wherein either ring is optionally substituted with a
group selected from halogen, alkyl, hydroxyalkyl,

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carboxyl, carboxyalkyl, carboxyalkylthio, thiocyanato, amino, hydrazino and a conjugating group for coupling a targeting molecule to said either ring.

5 According to another aspect of the invention, the chelators of the above formula are provided in a form having the metal radionuclide complexed therewith.

In another aspect of the invention, there is provided a conjugate in which the chelator is provided in
10 a form coupled to a diagnostically useful targeting molecule, and optionally in combination with a complexed metal radionuclide, for imaging use.

Another aspect of the invention provides a process for preparing chelators of the invention that are coupled
15 to a diagnostically useful targeting molecule. In particular, the method employs solid phase peptide synthesis techniques to prepare chelator conjugates.

In another aspect of the invention, there is provided methods of imaging sites of inflammation with
20 chelators of the invention coupled to a diagnostically useful targeting molecule in a form complexed with a traceable metal. In particular the targeting molecule is a peptide that localizes at sites of inflammation by binding to a particular receptor.

25 In yet another aspect of the invention, there is provided a method of imaging a site of inflammation within a mammal. The method involves the step of administering a compound that contains a diagnostically effective amount of a chelator of the invention coupled
30 to a diagnostically useful targeting molecule in a form complexed with a traceable metal.

Detailed Description of the Invention

The invention provides metal radionuclide chelators
35 that when conjugated to a targeting molecule are useful for delivering a radionuclide to a body site of therapeutic or diagnostic interest. As illustrated in

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the above formula, the chelators are hydrazino-type compounds that present an N₃S configuration in which the radionuclide is complexed.

- Terms defining the variables R¹ - R¹⁰ as used hereinabove have the following meanings:
- 5 "alkyl" refers to a straight or branched C₁-C₈ chain and embraces the term "lower alkyl" which refers to a straight or branched C₁-C₃ chain;
 - "halogen" refers to F, Cl and Br;
 - 10 "sulfur protecting group" refers to a chemical group that inhibits oxidation, including those that are cleaved upon chelation of the metal. Suitable sulfur protecting groups include known alkyl, aryl, acyl, alkanoyl, aryloyl, mercaptoacyl and organothio groups.
 - 15

- In preferred embodiments of the invention, the chelators conform to the above formula in which:
- R¹ is a hydrogen atom or a sulfur protecting group selected from benzoyl, acetamidomethyl and substituted or unsubstituted tetrahydropyranyl groups;
 - 20 R² and R³ are selected independently from H and a lower alkyl group selected from ethyl, propyl and most preferably methyl;
 - R⁴ and R⁷ are each H;
 - 25 R⁵ and R⁶ are selected independently from H, carboxyl and lower alkyl which is preferably methyl; and R⁸ is selected from H, carboxyl, and lower alkyl, preferably methyl.

- In specific embodiments of the invention, the chelators conform to the above general formula wherein R¹ is H or a sulfur protecting group and R² through R⁸ are each H. Particular chelators include:
- N(-S-benzoylmercaptoacetyl)-glycyl-2-pyridylhydrazide
 - N(-S-benzoylmercaptoacetyl)-L-glutamyl-(gamma-methyl ester)-2-pyridylhydrazide
 - 35 N(-S-benzoylmercaptoacetyl)-glycyl-3-(6-chloropyridazyl)-hydrazide

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N- (S-benzoylmercaptoacetyl) -L-glutamyl- (gamma-methylester) -3- (6-chloropyridazyl) hydrazide
N(-S-benzoylmercaptoacetyl)-glycyl-6-hydrazino nicotinic acid

5 N- (S-benzoylmercaptoacetyl) -glycyl-6-hydrazino nicotinyl-N-hydroxysuccinimide

N- (S-benzoylmercaptoacetyl) -glycyl-2-pyrimidylhydrazide

The substituents represented by R⁹ and R¹⁰ together with the adjacent nitrogen atom form a 5- or 6-membered, 10 saturated or unsaturated heterocyclic ring which may be fused to another five or six membered saturated or unsaturated, heterocyclic or carbocyclic ring. Five and six membered heterocyclic rings include but are not limited to pyrole, pyrazole, imidazole, pyridine, 15 pyrazine, pyridazine, pyrimidine and triazine. Fused rings include but are not limited to N-containing bicyclics such as quinoline, isoquinoline, indole and purine. Rings containing sulfur atoms e.g. thiazole, and rings containing oxygen atoms e.g. oxazole are also 20 encompassed by the present invention.

Suitable substituents on the heterocyclic ring formed by R⁹ and R¹⁰ include from 1-3 groups selected from halogen, carboxyl, carboxy-alkylthio and C₁₋₈ alkyl optionally substituted with 1-3 groups selected from 25 hydroxyl, carboxyl, thiocyanato, amino and hydrazino groups.

For coupling to a targeting molecule, R⁹ and R¹⁰ desirably incorporate a conjugating group. Conjugating groups are chemically reactive groups that allow for 30 coupling of the chelator to a targeting molecule. In the preferred case where the targeting molecule is a peptide or protein, the conjugating group is reactive under conditions that do not denature or otherwise adversely affect its targeting properties. In one embodiment of 35 the invention, the conjugating group is reactive with a functional group of the peptide/protein such as an amino terminal group or an ε-amino group of a lysine residue,

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so that the conjugating reaction can be conducted in a substantially aqueous solution. Useful conjugating groups include but are not limited to groups such as carboxyl, activated esters, carboxy-methylthiols,
5 thiocyanates, amines, hydrazines, maleimides, thiols, and activated halides. In a preferred embodiment of the invention, conjugating groups are selected from methyl propanoate, carboxyl group and N-hydroxysuccinimide ester. Carboxyl conjugating groups may be activated with
10 carbodiimide and an alcohol thereby forming an ester that is reactive with an amino group available on targeting molecules such as peptides and amino sugars, to form an amide linkage between the targeting molecule and the chelator conjugating group.

15 For diagnostic imaging purposes, the chelator per se may be used in combination with a metal radionuclide. Suitable radionuclides include technetium and rhenium in their various forms such as $^{99m}\text{TcO}^{3+}$, $^{99m}\text{TcO}_2^+$, ReO^{3+} and ReO_2^+ . More desirably, the chelator is coupled through
20 its conjugating group to a targeting molecule to form a conjugate that serves to deliver a chelated radionuclide to a desired location in a mammal. Examples of targeting molecules include, but are not limited to, steroids, proteins, peptides, antibodies, nucleotides and
25 saccharides. Preferred targeting molecules include proteins and peptides, particularly those capable of binding with specificity to cell surface receptors characteristic of a particular pathology. For instance, disease states associated with over-expression of
30 particular protein receptors can be imaged by labelling that protein or a receptor binding fragment thereof in accordance with the present invention. Representative peptides capable of specifically binding to target sites include:
35 atherosclerotic plaque

YRALVDTLK

RALVDTLK

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RALVDTLKFKVTQAE^{GAK}
YAKFRETLEDTRDRMY
AKFRETLEDTRDRMY
YAALDLNAVANKIADFEL
5 AALDLNAVANKIADFEL
YRALVDTLKFKVTEQAKGA
RALVDTLKFKVTEQAKGA
YRALVDTEFKVKQEAGAK
RALVDTEFKVKQEAGAK
10 YRALVDTLKFKVTQAE^{GAK}
infections and atherosclerotic plaque
VGVAPGVGVAPGVGVAPG
VPGVGVPGVGVPGVGVPGVG
formyl.Nleu.LF.Nleu.YK
15 formylMIFL
formylMLFK
formylMLFI
formylMFIL
formylMFLI
20 formylMLIF
formylMILF
formylTKPR
VGVAPG
formylMLF
25 YIGSR
CH₂CO.YIGSRC
thrombus
NDGDFEEIPEEYLQ
NDGDFEEIPEEY (SO₃Na) LQ
30 GPRG
platelets
D-Phe.PRPGGGGNGDFEEIPEEYL
RRRRRRRRRGDV
PLYKKIIKKLLES
35 RGD
RGDS
amyloid plaque (Alzheimer's disease)

EKPLQNFTLSFR

In a particular embodiment of the invention, imaging of inflammation is achieved using a conjugate in which the targeting molecule is a chemotactic peptide comprising the amino acid sequence Thr-Lys-Pro-Pro-Lys (TKPPR). It has been found that this peptide binds particularly well to "Tuftsin" receptors on leukocytes. Targeting peptides can be spaced from the chelator or the conjugating group by additional amino acid residues, preferably glycine, provided the peptide retains its localizing activity. In a particular embodiment, the TKPPR peptide is coupled to chelators of the invention by a Gly residue coupled to the conjugating group.

Peptide-based targeting molecules can be made, either per se or as chelator conjugates, using various established techniques. Because it is amenable to solid phase synthesis employing alternating t-Boc protection and deprotection is the preferred method of making short peptides. Recombinant DNA technology is preferred for producing proteins and long fragments thereof.

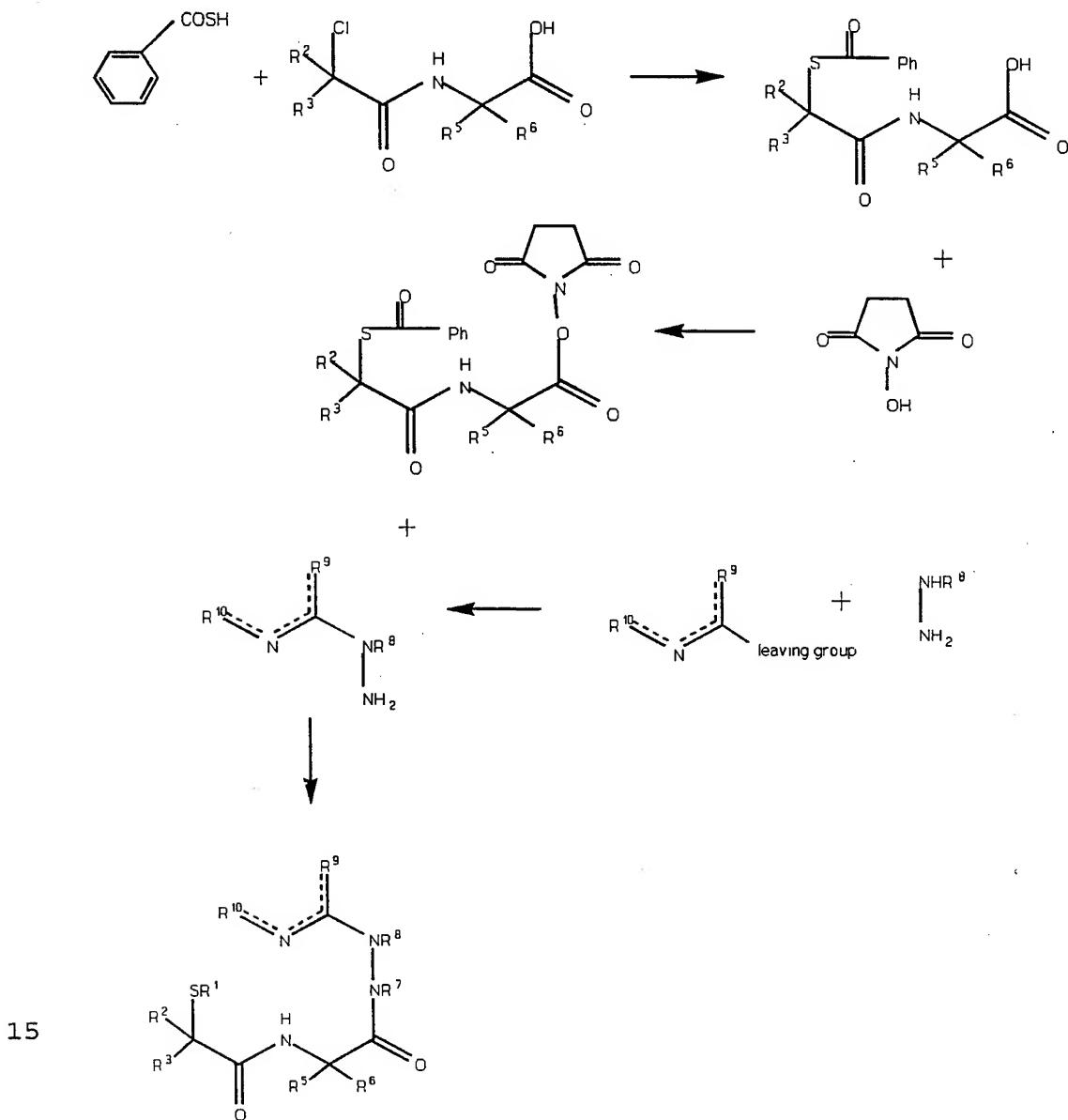
Specific chelators of the present invention are those in which R⁹ and R¹⁰ form a 6-membered, N-containing heterocyclic ring. In a preferred embodiment, R⁹ and R¹⁰ form a pyridine ring attached to the hydrazide moiety at the number 2 position which is the carbon atom adjacent to the nitrogen atom. In another embodiment of the present invention, the ring formed by R⁹ and R¹⁰ incorporates a conjugating group such as N-hydroxysuccinimide or more preferably a carboxyl group.

Specific chelators of the present invention can be prepared by the following general procedure.

Commercially available N-chloroacetylglycine, or a variant thereof substituted as desired, is reacted with potassium thiobenzoate yielding N-benzoylthioacetyl-glycine. This intermediate is then transformed into the corresponding N-hydroxysuccinimide ester using dicyclo carbodiimide in dioxane. The ester is reacted in

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dioxane at room temperature with a selected hydrazino substituted ring obtained by reacting the ring with hydrazine. It is understood that the hydrazino substituents at the carbon atom adjacent to the coordinating nitrogen atom of the ring are obtained when the ring is substituted at that position with a suitable leaving group, such as chlorine, prior to the reaction with hydrazine. The resulting chelator is then purified by recrystallization. A method of preparing the present chelators is represented below:



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It is to be understood that the present invention encompasses various heterocyclic compounds that contain a nitrogen atom adjacent to the carbon atom attached to the hydrazide substituent. For example, nicotinic acid when reacted with hydrazine yields 6-hydrazino nicotinic acid used to synthesize a metal chelator with a carboxyl group for conjugating a targeting molecule thereto. Other possible rings include but are not limited to five membered rings, bicyclics as well as rings containing additional nitrogen atoms or sulfur or oxygen atoms.

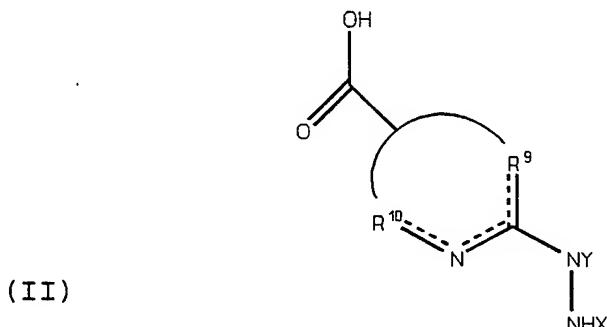
Further, it is to be understood that variation at R² and R³ can be introduced by using derivatives of N-chloroacetyl-glycine. For example, the acetyl portion may have one or two substituents such as lower alkyl (C₁₋₄) that may be substituted with from 1-3 groups selected from hydroxyl and carboxyl. Variation at R⁵ and R⁶ may be introduced by incorporating any N-chloro D or L amino acid (except proline) in place of glycine or by using glycine substituted with for instance C₁₋₈alkyl, hydroxyl, carboxyl group. Alkyl substituents at R⁵ and R⁶ may be straight or branched and optionally substituted with from 1-3 halogen atoms, carboxyl, mercapto, amino or aminocarbonyl groups.

In a particularly preferred embodiment, chelator-peptide conjugates are prepared by solid-phase peptide synthesis methods, which involve the stepwise addition of amino acid residues to a growing peptide chain that is linked to an insoluble (solid) support or matrix, such as polystyrene. The C-terminus residue of the targeting peptide is first anchored to a commercially available support with its amino group protected with an N-protecting agent such as a fluorenylmethoxycarbonyl (FMOC) group. The amino protecting group is removed with suitable deprotecting agents such as piperidine and the next amino acid residue (in N-protected form) is added with a coupling agent such as dicyclophosphodiimide (DCC). Upon formation of a peptide bond, the reagents are washed

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from the support. After addition of the final residue, the chelator is coupled to the N-terminus of the targeting peptide by the addition first of a synthetically prepared residue of the formula

5



10

wherein X is H or an aminoprotecting group and Y is C₁-alkyl and then adding both an amino acid residue of the formula -C(O)-C(R⁵R⁶)-NHX and mercaptoacetic acid or a derivative of the formula -C(O)-C(R²R³)-SR¹, where R¹, R², R³, R⁵ and R⁶ are as defined above. It will be appreciated that the chelator may be coupled to the N-terminus of the targeting peptide as a whole or in part ie. where X of the synthetically prepared residue is -C(O)-C(R⁵R⁶)-NHZ or -C(O)-C(R⁵R⁶)-NH-C(O)-C(R²R³)-SR¹ wherein Z is H or an aminoprotecting group. The synthetic residue of formula (II) is prepared from an N-containing heterocycle which is derivatized with a carboxyl conjugating group and a leaving group adjacent to the coordinating nitrogen atom, for example, commercially available 6-chloronicotinic acid. This is reacted with N-alkyl-hydrazine to form an N-alkyl-hydrazino substituted heterocycle. In a particular embodiment N-methylhydrazine is reacted with 6-chloronicotinic acid to give 6-(N-methyl-hydrazino)nicotinic acid which is employed in the solid phase synthesis as an individual amino acid residue. Following the addition of 6-(N-methylhydrazino)nicotinic acid to the targeting peptide another amino acid residue

according to the formula $-C(O)-C(R^5R^6)-NHZ$ is coupled to the N'-amino group and finally a mercapto acetic acid derivative of the formula $-C(O)-C(R^2R^3)-SR^1$. After addition of the mercaptoacetic acid residue to the chain 5 the, chelator-conjugate is cleaved from the support with a suitable reagent such as trifluoroacetic acid (TFA).

In a most preferred embodiment of the invention a chelator conjugate is prepared on a solid support and has the structure of formula (I) wherein the targeting 10 molecule is a peptide having a sequence Gly-Thr-Lys-Pro-Pro-Arg-OH, the conjugating group is a carboxyl group substituent on a pyridine ring formed by R⁹ and R¹⁰, R⁸ is methyl, R⁷ is H, one of R⁵ and R⁶ is H and the other is hydroxymethyl, R⁴ is H, R³ and R² are both H and R¹ is the 15 sulfur protecting group acetamidomethyl.

Incorporation of the selected radionuclide within the chelator can be achieved by various methods. A chelator solution is formed initially by dissolving the chelator in aqueous alcohol eg. ethanol-water 1:1. 20 Oxygen is removed for example by degassing with N₂, then sodium hydroxide is added to remove the thiol protecting group. The solution is again purged of oxygen and heated on a water bath to hydrolyze the thiol protecting group, and the solution is then neutralized with an organic acid 25 such as acetic acid (pH 6.0-6.5). In the labelling step, sodium pertechnetate is added to the chelator solution with an amount of stannous chloride sufficient to reduce the technetium. The solution is mixed and left to react at room temperature and then heated on a water bath. In 30 an alternative method, labelling can be accomplished with the chelator solution adjusted to pH 8. At this higher pH, pertechnetate may be replaced with a solution containing technetium complexed with labile ligands suitable for ligand exchange reactions with the desired 35 chelator. Suitable ligands include tartarate, citrate and heptagluconate. Stannous chloride may be replaced with sodium dithionite as the reducing agent if the

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chelating solution is alternatively adjusted to a still higher pH of 12-13 for the labelling step. The chelators of the present invention can be coupled to a targeting molecule prior to labelling with the radionuclide, a 5 process referred to as the "bifunctional chelate" method. An alternative approach is the "prelabelled ligand" method in which the chelator is first labelled with a radionuclide and is then coupled to the targeting molecule.

10 The labelled chelator may be separated from contaminants $^{99m}\text{TcO}_4^-$ and colloidal $^{99m}\text{TcO}_2$ chromatographically, e.g., with a C-18 Sep Pak column activated with ethanol followed by dilute HCl. Eluting with dilute HCl separates the $^{99m}\text{TcO}_4^-$, and eluting with 15 EtOH-saline 1:1 brings off the chelator while colloidal $^{99m}\text{TcO}_2$ remains on the column.

When coupled to a targeting molecule and labelled with a diagnostically useful metal, chelators of the present invention can be used to detect pathological 20 conditions by techniques common in the art of diagnostic imaging. A chelator/targeting molecule conjugate labelled with a radionuclide metal such as technetium may be administered to a mammal intralymphetically, intraperitoneally and preferably intravenously in a 25 pharmaceutically acceptable solution such as saline or blood plasma medium. The amount of labelled conjugate administered is dependent upon the toxicity profile of the chosen targeting molecule as well as the profile of the metal and is generally in the range of about 0.01 to 30 100mCi/70Kg and preferably 10 to 50mCi/70Kg host. Localization of the metal in vivo is tracked by standard 35 scintigraphic techniques at an appropriate time, typically at regular intervals between 15 minutes and 24 hours subsequent to administration. In a particular embodiment, chelators of the invention coupled to the peptide TKPPR in a saline solution are administered to a mammal by intravenous injection to image sites of focal

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inflammation.

The following examples are presented to illustrate certain embodiments of the present invention.

- 5 Example 1 - Preparation of N-(S-benzoylmercaptoacetyl)-glycine
To a stirring solution under argon at 0°C of (1.50g, 10mmoles) N-chloroacetyl glycine in (25mL) ethanol was added (1.41ml, 12mmoles) thiobenzoic acid, followed by (7.0mL, 3N, 21mmoles) potassium hydroxide over 3 minutes.
10 The reaction was allowed to heat to room temperature for 20 minutes followed by reflux under argon at 50°C for 2 hours. The reaction was cooled to room temperature and acidified with (5mL, 2N) hydrochloric acid. The ethanol
15 was rotavapped off to leave N-(S-benzoylmercaptoacetyl)-glycine (m.p. 140.5-141.5°C), a white solid, which was washed with water, filtered, and dried in vacuo (2.46g, 98% yield).

20 Example 2- Preparation of chelator N-(S-benzoylmercaptoacetyl)-glycyl-2-pyridylhydrazide
To a stirring solution at 7°C of (1.10g, 4.35mmoles) N-(S-benzoylmercaptoacetyl)-glycine and (502mg, 4.35mmoles) N-hydroxysuccinimide in (34mL) dioxane was added a solution of (904mg, 4.38mmoles) DCC in (1mL) dioxane. After the reaction stirred for 5 hours at room temperature it was stored overnight at 4°C. It was then filtered and rotavapped to a white solid. N-(S-benzoylmercaptoacetyl)-glycine N-hydroxy succinimide ester, was triturated with cold isopropanol, filtered, and dried in vacuo to a white solid (1.40g, 92% yield).
30 To a stirring solution of (1.50g, 4.45mmoles) N-(S-benzoylmercaptoacetyl)-glycyl N-hydroxysuccinimide ester in (30mL) dioxane under argon was added a solution of (500mg, 4.45mmoles) 2-hydrazinopyridine in (6mL) dioxane. After 1/2 hour dioxane was stripped off to a yellow oil. The

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product (1.46g, 99%, m.p. 162-162.5°C) solidified in (10mL) aqueous sodium bicarbonate. The product, N(-S-benzoylmercaptoacetyl)-glycyl-2-pyridylhydrazide, was suction filtered and washed with water and then (700mg) of 5 product was recrystallized from (1:1) ethyl acetate:dioxane (600 mg, 85%).

Example 3 - Preparation of chelator N-(S-benzoylmercaptoacetyl)-L-glutamyl-(gamma-methyl ester)- 2-pyridylhydrazide

To a stirring solution of (2.73g, 9.31mmoles) N-(S-benzoylmercaptoacetyl)-glycyl N-hydroxysuccinimide ester in (50mL) dioxane was added a solution of (1.50g, 9.31mmoles) L-glutamic acid methyl ester and (1.88g, 18.6mmoles) 10 triethylamine in (50mL) dioxane. After 7 hours the dioxane was stripped off and (10mL) water was added. The solution was acidified to pH 2.5, extracted into ethyl acetate, dried over sodium sulfate, filtered, and rotavapped to 15 yield a pale yellow oil of

20 N-(S-benzoylmercaptoacetyl)-L-glutamic acid methyl ester.

To a stirring solution at room temperature of (9.3mmole) N-(S-benzoylmercaptoacetyl)-L-glutamic acid methyl ester and (1.07g, 9.31mmoles) N-hydroxysuccinimide in (100mL) dioxane was added a solution of (1.92g, 25 9.31mmoles) DCC in (25mL) dioxane. After the reaction stirred for 7 hours it was filtered, then rotavapped to a white solid. N-(S-benzoylmercaptoacetyl)-L-glutamyl-(gamma-methyl ester). N-hydroxysuccinimide ester, was triturated with cold isopropanol, filtered and dried in vacuo to a 30 white fluffy solid (3.70g, 91% yield).

To a stirring solution of (1.00g, 2.29mmoles) N-(S-benzoylmercaptoacetyl)-glutamyl-(gamma-methyl ester) N-hydroxysuccinimide ester in (20mL) dioxane under argon was added a solution of (250mg, 2.29mmoles) 2-hydrazinopyridine 35 in (10mL) dioxane. After 1 hour dioxane was stripped off to pale yellow oil. The product, N(-S-benzoylmercaptoacetyl)-L-glutamyl-(gamma-methyl ester)- 2-pyridylhydrazide (760mg,

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77%) solidified in (5mL) water. The product (m.p. 162-163°C) was suction filtered and washed with water and then recrystallized from (3:1) ethyl acetate: dioxane (200mg).

5 Example 4 - Preparation of chelator N-(S-benzoylmercaptoacetyl)-glycyl-3-(6-chloropyridazyl)-hydrazide

A solution of (6.0g, 40.3mmoles) 3,6-dichloropyridazine and (4.0g, 80mmoles) hydrazine monohydrate in (50mL) ethanol
10 was refluxed under argon for 3 hours. The solution was cooled to room temp. where it solidified to an off-white solid matrix. 6-chloro-3-hydrazinopyridazine was crushed, washed in (20mL) water, filtered and then recrystallized from hot water to a white spongy crystal.

15 To a stirring solution of (845mg, 2.50mmoles) N-(S-benzoylmercaptoacetyl)-glycyl N-hydroxysuccinimide ester in (30mL) dioxane under argon was added (361mg, 4.45mmoles) 6-chloro-3-hydrazinopyridazine which slowly dissolved. After 40 minutes a white crystal formed. The product (m.p. 164-
20 165.5°C) was suction filtered and washed with a small portion of dioxane and then recrystallized from ethyl acetate: methanol (200mg).

25 Example 5 - Preparation of chelator N-(S-benzoylmercaptoacetyl)-L-glutamyl-(gamma-methylester)-3-(6-chloropyridazyl)-hydrazide

A solution of (6.0g, 40.3mmoles) 3,6-dichloropyridazine and (4.0g, 80mmoles) hydrazine monohydrate in (50mL)
30 ethanol was refluxed under argon for 3 hours. The solution was cooled to room temp. where it solidified to an off-white solid matrix. 6-chloro-3-hydrazinopyridazine was crushed, washed in (20mL) water, filtered, and then recrystallized from hot water to a white spongy crystal.

35 To a stirring solution at room temperature of (2.0g, 4.58mmoles) N-(S-benzoylmercaptoacetyl)-L-glutamyl-(gamma-methyl ester) N-hydroxysuccinimide ester in (25mL)

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dioxane was added a partially dissolved solution of (662mg, 4.58mmoles) 3-(6-chloropyridazyl)-hydrazide in (15mL) dioxane. After the reaction stirred for 1 hour all starting material had dissolved. The dioxane was
5 rotavapped off to a pale yellow oil. After (5mL) water was added the oil formed a pale yellow solid. The product (m.p. 164-165) was filtered, washed with water, and dried in vacuo, (1.80g, 85% yield).

10 Example 6 - Preparation of chelator N-(S-benzoylmercaptoacetyl)-glycyl-6-hydrazino nicotinic acid

A solution of (3.00g, 19mmoles) 6-chloronicotinic acid and (4.62mL, 95mmoles) hydrazine monohydrate in (15mL)
15 ethanol was refluxed under argon for 18 hours. Upon cooling to room temperature a white solid product, 6-hydrazino nicotinic acid, formed. This was filtered and washed with ethanol then dissolved in water, acidified to pH 7.0 with acetic acid causing a white precipitate. This
20 was filtered, washed with water, and dried in vacuo over KOH, (1.87g, 64% yield).

To a stirring solution of (460mg, 3.0mmoles) 6-hydrazinonicotinic acid and (318mg, 3.0mmoles) sodium carbonate in (15mL) water was dripped a solution of (1.02g, 3.0mmoles) N-(S-benzoylmercaptoacetyl)-glycyl N-hydroxysuccinimide ester in (25mL) dioxane. The reaction began immediately with a yellow color forming. After 1 hour dioxane was rotavapped off to leave (10ml) water. This was acidified to pH 5.5 with 2N hydrochloric acid
25 causing a white precipitate to form. The product was filtered, washed with water, and dried in vacuo, (902mg, 80% yield). Melting point was not obtained as the product decomposed over a large range.

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Example 7 - Preparation of chelator N-(S-benzoylmercaptoacetyl)-glycyl-6-hydrazino nicotinyl-N-hydroxy succinimide

To a stirring solution of (376mg, 1.0mmoles) N-(S-benzoylmercaptoacetyl)-glycyl-6-hydrazinonicotinic acid and (115.3mg, 1.0mmoles) N-hydroxysuccinimide ester in (30mL) dioxane was added drop-wise a solution of (206mg, 1.0mmole) dicyclo carbodiimide in (5mL) dioxane. The solution was filtered then rotavapped to give a pale yellow semi-solid oil which was triturated with cold isopropanol to give a pale yellow solid. The product was filtered, rinsed with isopropanol, and dried in vacuo. The melting point was found to be 176-178°C followed by decomposition.

15

Example 8 - Preparation of chelator N-(S-benzoylmercaptoacetyl)-glycyl-2-pyrimidylhydrazide

To a stirring solution of (1.00g, 8.73mmoles) 2-chloropyrimidine in (3mL) dioxane under argon was added (1.31g, 26.2mmoles) hydrazine monohydrate. The reaction started immediately giving off heat. The solution was refluxed under argon at 60°C for 2 hours. Upon cooling to room temperature a white solid crystallized out. A mixture of (1:1, 20mL) dioxane:water was added dissolving the solid. The solvents were rotavapped to about 2ml of liquid and a white solid. 2-hydrazinopyrimidine (955mg, 99% yield) was filtered, washed with dioxane, and dried in vacuo.

30

To a stirring solution of (200mg, 1.82mmoles) 2-hydrazinopyrimidine in (25mL) dioxane was added a solution of (615mg, 1.82mmoles) N-(S-benzoylmercaptoacetyl)-glycyl-N-hydroxysuccinimide ester in (25mL) dioxane. The reaction was stirred for 1 hour and the dioxane was rotavapped off to a yellow oil. This was dissolved in isopropanol and rotavapped to a white solid. The solid product (m.p. 176-178°C) was washed with

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aqueous bicarbonate, filtered and dried in vacuo, (510mg, 84% yield). A small amount (120mg) was recrystallized from hot methanol.

5 Example 9 - Preparation of 99m Tc labelled chelators
 The chelator N-(S-benzoylmercaptoacetyl)-glycyl-2-pyridylhydrazide (example 2, 1mg) was dissolved in EtOH-water and hydrolyzed by heating with NaOH, after which the reaction mixture was neutralized with acetic acid to
 10 pH 6. 350 μ g hydrolyzed chelator was reacted with 103 MBq TcO₄⁻ and 10 μ g SnCl₂, at room temperature. Approximately half activity was present as TcO₂ which did not change after heating 10 minutes at 80°C. To achieve adequate separation of the chelator, the solution was then loaded
 15 on a C-18 Sep-Pak column which was eluted with dilute HCl to remove TcO₄⁻. Subsequent elution with 1:1 EtOH-saline removed the chelator while TcO₂ remained on the column.

Another chelator was similarly labelled.
 Particularly, 300 μ g of the hydrolyzed chelator N-(S-benzoylmercaptoacetyl)-glycyl-3-(6-chloropyridazyl)-hydrazide (example 4) was adjusted to pH 12-13 and reduced with 100 μ g of sodium dithionite for 10 minutes at 75°C. The resulting mixture was then loaded on a C-18 Sep-Pak column and eluted with dilute HCl followed by
 25 EtOH-saline. The extent of complexation of 99m Tc with chelators was measured by radioactivity of the eluted fractions; results are shown in the table below.

Example 2 Example 4

30

TcO ₄ ⁻ (HCl eluate)	0.15 (1%)	1.38 (8%)
TcO ₂ (column)	12.5 (31%)	2.55 (14%)
Chelator (EtOH-saline eluate)	27.6 (68%)	14.19 (78%)

35 * units of measure in MBq with corresponding percent of total

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Example 10 - In vivo distribution

Distribution within rats of selected chelators and a reference chelator was determined using established protocols. Briefly, male Wistar rats (Charles River, 5 200g) were anesthetized with somnitol (40 to 50mg/kg) and 200 μ L of the labelled chelator (ie. 200 μ Ci) was injected intravenously via the tail vein. Serial whole-body scintigrams were acquired for first 10 minutes. After further images were obtained at 60 and 120 minutes, the 10 rat was killed with anesthesia and samples of organs (blood, heart, lung, liver, spleen, kidney, muscle, GI tract) were weighed and counted in either a well-type gamma counter or in a gamma dose calibrator. Dose calculations were made based on the assumption that rats 15 weighed 200g and that the blood volume constituted 8% body weight. All results were corrected for the residual dose in the tail.

The chelators were found to clear relatively rapidly from the blood as desired. For the chelator of example 20 2, it was found that the liver and GI tract accounted for about 60% of the dose, with only about 6% remaining in the blood. The chelator of example 5 localized primarily (55%) in the GI tract, with only about 10% of the dose remaining in the blood. About 70% of the chelator of 25 example 4 was localized in the liver and GI tract.

Of these chelators, the chelator of example 4 showed the fastest clearance from the blood and other tissues and is mainly eliminated through the liver and GI tract. The chelator of example 3 had the greatest accumulation. 30 in the kidney.

The reference chelator, lacking the hydrazide-based structure yet having the N₃S configuration N-(S-benzoylmercaptoacetyl)-glycyl-2-pyridylmethylamide, was extensively retained in the blood pool which would be 35 disadvantageous for imaging as this contributes to higher levels of the radionuclide in all tissues.

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Example 11 - Preparation of Chelator-Peptide Conjugate
S-Acm-Mercaptoacetyl-Ser-N-methylhydrazino
nicotinic acid- Gly-Thr-Lys-Pro-Pro-Arg

The title chelator conjugate was prepared by solid phase
5 peptide synthesis using Fmoc chemistry on an Fmoc-
arginine preloaded 2-methoxy-4-alkoxybenzyl alcohol resin
(Sasrin Resin, Bachem Biosciences Inc., Philadelphia) with
an Applied Biosystems 433A peptide synthesizer (Foster
City, CA). The synthetically prepared residue 6-(N-
10 methylhydrazino)nicotinic acid and commercially obtained
acetamidomethyl-mercaptopropionic acid were incorporated in
the peptide by coupling to the Gly and Ser residues
respectively.

Synthetic residue 6-(N-methylhydrazino)nicotinic
15 acid was prepared as follows. To a solution of (2.0g,
12.69mmole) 6-chloronicotinic acid in (15mL) ethanol was
added (1.38mL, 26 mmole) N-methylhydrazine. The reaction
was refluxed under argon for 2 days where the resulting
precipitate, 6-(N-methylhydrazino)nicotinic acid, was
20 collected, washed with 10mL ethanol, and dried in vacuo
to a white solid (1.34g, 63.3%).

The N'-amino group of 6-(N-methylhydrazino)nicotinic
acid was Fmoc protected as follows. To a solution of
(500mg, 2.99mmole) 6-(N-methylhydrazino)nicotinic acid in
25 (1N, 45mL) aqueous potassium carbonate and (55mL) dioxane
at 0°C was added dropwise a solution of (1.22g, 4.71mmole)
Fmoc-chloride in 5mL dioxane. The solution was warmed to
room temp. and stirred for 8 hours and then rotavapped to
30mL. 50mL water was added to the solution and then
30 washed with (2x40mL) diethyl ether. The aqueous phase
was acidified with 1N HCl to pH 5 and the solution was
extracted with (3X50mL) ethyl acetate and 6-(N'-Fmoc, N-
methylhydrazino)nicotinic acid was dried over MgSO₄,
filtered and rotavapped to an off white solid (680mg,
35 73.5%).

The peptide-resin was dried under vacuum overnight
and cleavage of the peptide from the resin was achieved

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- by mixing a cooled solution of 9.5mL trifluoroacetic acid (TFA), 0.5mL water, 0.5mL thioanisole and 0.25mL 2-ethanedithiol (1mL per 100mg of peptide-resin) with the peptide-resin for 1.5 to 2 hours at room temperature.
- 5 The resin was removed by filtration and washed with 1-3mL of TFA to obtain 6-8mL of a clear yellow liquid. This liquid was slowly dropped into 30-35mL of cold tert-butyl ether in a 50mL conical polypropylene centrifuge tube forming a white precipitate. The precipitate was
- 10 centrifuged at 7000rpm, 0°C for 5 minutes (Sorvall RT6000, Dupont), decanted and washed two more times with tert-butyl ether. Following drying under vacuum the precipitate was dissolved in water. The precipitate was frozen in acetone-dry ice and lyophilized overnight. The
- 15 resulting white powder was dissolved in water, filtered through a 0.45µm syringe filter (Gelman Acrodisc 3 CR PTFE), and purified by reversed-phase HPLC (Beckman System Gold) with a C18 column (Waters RCM 25 x 10) using 1% TFA in water as buffer A and 1% TFA in acetonitrile as
- 20 buffer B. The column was equilibrated with 100:0 buffer A:buffer B and eluted with a linear gradient in 25 minutes at 1mL/min to 50% buffer B. Fractions were reanalysed on the HPLC and pooled according to matching profiles. The pure fractions were frozen in acetone-dry
- 25 ice and lyophilized overnight to give a white powder.

Example 12 - Labelling of Chelator-Conjugate S-Acm-Mercaptoacetyl-Ser-N-methylhydrazino nicotinic acid-Gly-Thr-Lys-Pro-Pro-Arg

- 30 The chelator-conjugate of example 11 (200µL, 1mg/mL saline) was injected into a 3mL vacutainer with 100µL pertechnetate (10mCi) and 100µL stannous gluconate (50 µg stannous chloride and 1 mg sodium gluconate). The tube was placed in a boiling water bath for 12 minutes and
- 35 then filtered through a Watman PVDF syringe filter to collect the labelled conjugate solution which was further diluted with saline to prepare an injectable solution

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(2Mboq/mL). The chelator-conjugate was isolated by HPLC (Beckman) from a (20 μ L) sample (before dilution) to determine the labelling yield by measuring radioactivity of the fractions. 70.8%, 5.1% and 15.0% of the label was 5 found in each of three fractions eluted for a 90.9% total labelling yield.

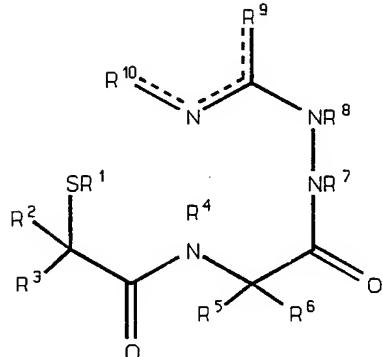
Although preferred embodiments of the invention are described herein in detail, it will be understood by those skilled in the art that variations may be made 10 thereto without departing from the spirit of the invention or the scope of the appended claims.

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WE CLAIM:

1. A compound of the general formula:

5



10

wherein

R¹ is H or a sulfur protecting group;

15 R² and R³ are selected independently from H; carboxyl; lower alkyl; and lower alkyl substituted with a group selected from hydroxyl, sulfhydryl, halogen, carboxyl and aminocarbonyl;

20 R⁴ and R⁷ are each H;

R⁵ and R⁶ are selected independently from H; carboxyl; lower alkyl; lower alkyl substituted with a group selected from hydroxyl, sulfhydryl, halogen, carboxyl, lower

25 alkoxy carbonyl and aminocarbonyl; and an alpha carbon side chain of any amino acid other than proline;

R⁸ is selected from H, carboxyl, lower alkyl and lower alkyl substituted with hydroxyl, carboxyl or halogen; and

30 R⁹ and R¹⁰ together form a 5- or 6-membered, saturated or unsaturated heterocyclic ring which is optionally fused to another 5- or 6-membered saturated or unsaturated, heterocyclic or carbocyclic ring wherein either ring is optionally substituted with a group selected from halogen, alkyl, hydroxyalkyl, carboxyl,

35

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carboxyalkyl, carboxyalkylthio, thiocyanato, amino, hydrazino and a conjugating group for coupling a targeting molecule to said either ring.

5

2. A compound according to claim 1 wherein R², R³, R⁴, R⁵, R⁷ and R⁸ are hydrogen.
- 10 3. A compound according to claim 2 wherein R⁹ and R¹⁰ together form a pyridine ring.
- 15 4. A compound according to claim 2 wherein R⁹ and R¹⁰ together form a pyrimidine ring.
- 15 5. A compound according to claim 2 wherein R⁹ and R¹⁰ together form a 3-chloro substituted pyridazine ring.
- 20 6. A compound according to claim 2 wherein R⁹ and R¹⁰ together form a 5-carboxy substituted pyridine ring.
- 25 7. A compound according to claim 2 wherein R⁹ and R¹⁰ together form a 5-N-hydroxysuccinimide carboxy substituted pyridine ring.
8. A compound according to claim 2 wherein R⁹ and R¹⁰ together form a 3-chloro substituted pyridazine ring and R₆ is the group CH₂CH₂COOMe.
- 30 9. A compound according to claim 2, wherein R⁹ and R¹⁰ together form a pyridine ring and R₆ is the group CH₂CH₂COOMe.
- 35 10. A compound according to claim 1, wherein R¹ is selected from the group consisting of a hydrogen atom, benzoyl group, acetamidomethyl group and a substituted or unsubstituted tetrahydropyranyl

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group.

11. A compound according to claim 1, wherein R⁹ and R¹⁰ form a ring substituted by a conjugating group for coupling a targeting molecule to said other ring.
5
12. A compound according to claim 11, wherein the conjugating group is selected from the group consisting of carboxyl, N-hydroxysuccinimide ester
10 and methyl propanoate.
13. A compound according to claim 11, wherein R², R³, R⁴, R⁵, R⁷ and R⁸ are hydrogen.
- 15 14. A compound according to claim 13, wherein the ring formed by R⁹ and R¹⁰ is a six membered heterocyclic ring.
20
15. A compound according to claim 13, wherein R⁹ and R¹⁰ together form a 3-chloro substituted pyridazine ring.
25
16. A compound according to claim 13, wherein R⁹ and R¹⁰ together form a 5-carboxy substituted pyridine ring.
30
17. A compound according to claim 13, wherein R⁹ and R¹⁰ together form a 5-N-hydroxysuccinimide carboxy substituted pyridine ring.
35
18. A compound according to claim 13, wherein R⁹ and R¹⁰ together form a 3-chloro substituted pyridazine ring and R⁶ is the group CH₂CH₂COOMe.
19. A compound according to claim 13 wherein R⁹ and R¹⁰ together form a pyridine ring and R⁶ is the group CH₂CH₂COOMe.

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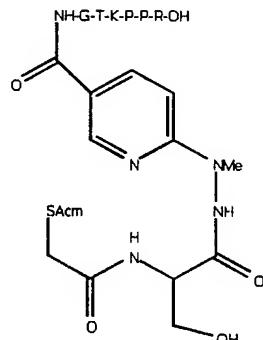
20. A compound according to claim 11, wherein R¹ is selected from the group consisting of a hydrogen atom, benzoyl group, acetamidomethyl group or a substituted or unsubstituted tetrahydropyranyl group.
- 5
21. A compound according to claim 11, wherein a targeting molecule is coupled to said conjugating group.
- 10
22. A compound according to claim 21, wherein the targeting molecule is a peptide.
- 15
23. A compound according to claim 1, in a form complexed with a metal radionuclide or an oxide or nitride thereof.
- 20
24. A compound according to claim 23, in a form complexed with ^{99m}Tc or oxide thereof.
- 25
25. A compound according to claim 21, in a form complexed with a metal radionuclide or an oxide or nitride thereof.
26. A compound according to claim 25, in a form complexed with ^{99m}Tc or oxide thereof.
- 30
27. A compound according to claim 22, wherein the peptide comprises the sequence TKPPR.
28. A compound according to claim 27, wherein the conjugating group is a carboxyl group.

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29. A compound according to claim 28, having the formula

5

10



30. A compound according to claim 29, in a form complexed with a metal radionuclide or an oxide or nitride thereof.

15

31. A compound according to claim 30, wherein said metal radionuclide is ^{99m}Tc .

20

32. A method of preparing a compound according to claim 22, wherein the conjugating group is a carboxyl group and R⁸ is C₁₋₄alkyl, the method comprising the step of coupling the targeting peptide to an intermediate of the formula

25

30

wherein

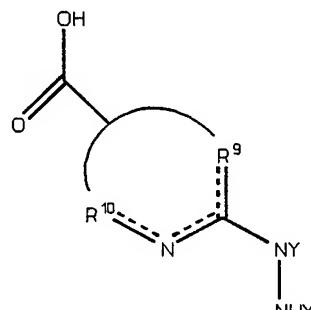
X is H, an

aminoprotecting group, -C(O)-C(R⁵R⁶)-NHZ, or
 $-C(O)-C(R^5R^6)-NH-C(O)-C(R^2R^3)-SR^1;$

35

Y is C₁₋₄alkyl; and

Z is H or an aminoprotecting group.



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33. The method according to claim 32, wherein the C-terminus of the targeting peptide is immobilized on a solid support.
- 5 34. A method of preparing a compound according to claim 29, comprising the step of coupling the targeting peptide NH-GTKPPR-OH to the intermediate of claim 32, wherein R⁹ and R¹⁰ form a pyridine ring and Y is methyl.
- 10 35. The method according to claim 34, wherein the C-terminus of the targeting peptide is immobilized on a solid support.
- 15 36. A method of imaging a site of inflammation within a mammal comprising the step of administering a diagnostically effective amount of a compound according to claim 27 in a form complexed with a metal radionuclide or an oxide or nitride thereof.
- 20 37. The method according to claim 36, wherein said metal radionuclide is ^{99m}Tc.
- 25 38. A method of imaging a site of inflammation within a mammal comprising the steps of administering a diagnostically effective amount of a compound according to claim 30.
- 30 39. The method according to claim 38, wherein said metal radionuclide is ^{99m}Tc.

INTERNATIONAL SEARCH REPORT

Inter. Application No
PCT/CA 94/00395

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 6 C07D213/77 A61K31/44 C07D213/80 C07D237/20 C07D239/42
 A61K31/465 A61K31/50 A61K31/505

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 IPC 6 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,91 09876 (NEORX CORPORATION) 11 July 1991 see the whole document ----	1-35
A	EP,A,0 384 769 (JOHNSON MATTHEY PUBLIC LIMITED COMPANY) 29 August 1990 -----	

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

4

Date of the actual completion of the international search 7 November 1994	Date of mailing of the international search report 17. 11. 94
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Authorized officer

De Jong, B

INTERNATIONAL SEARCH REPORTI national application No.
PCT/CA94/00395**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 36-39 are directed to a method of treatment of (diagnostic method practised on) the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/CA 94/00395

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9109876	11-07-91	US-A-	5112953	12-05-92
		EP-A-	0461245	18-12-91
		JP-T-	4505022	03-09-92
		US-A-	5175257	29-12-92
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EP-A-0384769	29-08-90	AU-B-	630668	05-11-92
		AU-A-	5007490	13-09-90
		CA-A-	2010800	24-08-90
		HU-B-	207293	29-03-93
		IL-A-	93432	27-02-94
		JP-A-	3027356	05-02-91
		US-A-	5206370	27-04-93
<hr/>				